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Health and Environmental Effects Profile for 3,3'-Dimethylbenzidine

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16. ABSTRACT

The Health and Environmental Effects Profile for 3,3'-Dimethylbenzidine was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. 3,3'-Dimethylbenzidine has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for 3,3'-dimethylbenzidine is 9.2 $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. Existing data are insufficient to determine an RQ value.

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR 3,3'-DIMETHYLBENZIDINE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
OFFICE OF HEALTH AND ENVIRONMENTAL ASSESSMENT
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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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EXECUTIVE SUMMARY

3,3'-Dimethylbenzidine is a white to reddish crystalline compound at ambient temperature. It is sparingly soluble in water and is soluble in ether, alcohol and dilute acids (Windholz, 1983). It is a weak base and undergoes reactions similar to those of benzidine (IARC, 1972; Ferber, 1978). 3,3'-Dimethylbenzidine is made by the reaction of o-nitrotoluene with zinc dust and caustic soda to form hydrazotoluene, which is subsequently rearranged by boiling with sulfuric or hydrochloric acid (Lurie, 1964; Powell et al., 1979). No current commercial manufacturers of 3,3'-dimethylbenzidine in the United States were reported in SRI (1986) or USITC (1985). In 1983, 75,307 pounds of this compound was imported into the United States through principal customs districts (USITC, 1984). It is estimated that >75% of the 3,3'-dimethylbenzidine consumed is used as an intermediate in the production of azo dyes and pigments used for coloring paper, textiles and leathers and for diverse applications in the petroleum, rubber, plastics, wood, soap, fur and hair dye industries (Powell et al., 1979; NIOSH, 1980). Approximately 20% of the 3,3'-dimethylbenzidine consumed domestically is used in the production of polyurethane-based high strength elastomers, coatings and rigid plastics and <5% is used for chlorine detection and other laboratory procedures (Powell et al., 1979).

3,3'-Dimethylbenzidine is a weak base and as such, may be protonated and form salts that are more soluble than the parent compound. Variations in the behavior of 3,3'-dimethylbenzidine in the environment may result from protonation. If released to water, 3,3'-dimethylbenzidine is expected to be oxidized by Fe(+3) and other cations fairly rapidly (U.S. EPA, 1979). The half-life of this oxidation may be <4 hours. It is likely to be resistant

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to hydrolysis (Schmitz and Rooze, 1981; Lyman et al., 1982). Potential exists for significant photolysis of 3,3'-dimethylbenzidine. Adsorption to microcrystalline clay and covalent binding to suspended solids and sediments may be significant (U.S. EPA, 1979; Parris, 1980). In fact, adsorption by microcrystalline clays may be the most important process in water. Bioaccumulation in aquatic organisms and volatilization from water should not be significant removal processes. If released to air, vapor phase 3,3'-dimethylbenzidine is predicted to react with hydroxyl radicals and has an estimated half-life of 4.0 hours at 25°C (U.S. EPA, 1986b). Direct photolysis in the troposphere is also likely to be a significant removal mechanism. Reaction with ozone is unlikely (U.S. EPA, 1986b). If released to soil, 3,3'-dimethylbenzidine is likely to be resistant to hydrolysis (Lyman et al., 1982). 3,3'-Dimethylbenzidine may be immobilized in soil by physical adsorption and covalent binding. Some mobilization may occur in acidic soils because of the protonation process. Volatilization from wet and dry soil surfaces should not be significant. Soil-catalyzed oxidation of 3,3'-dimethylbenzidine may be the most significant reaction of this chemical and the half-life of this reaction may be <1 day (U.S. EPA, 1980; Lapp et al., 1981).

Monitoring data regarding 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix. This compound is not known to occur naturally in the environment (U.S. EPA, 1980). Potential sources of major release are in air-borne particulate matter and wastewater from manufacturing and processing plants for dyes and pigments derived from 3,3'-dimethylbenzidine (U.S. EPA, 1980; Lapp et al., 1981).

Minor sources of release may originate from clothing, colored paper, textiles, leather and other dyed materials that are commonly disposed of in solid waste disposal sites (U.S. EPA, 1980).

The workers who manufacture and handle 3,3'-dimethylbenzidine-based dyes and pigments are likely to be the most exposed segment of the population (U.S. EPA, 1980). Urine samples from some workers exposed to azo dyes during manufacture (two sites) and use (four sites) contained ppb levels of 3,3'-dimethylbenzidine (Lowry et al., 1980). Workers in biochemical and clinical laboratories where 3,3'-dimethylbenzidine is used may also be exposed (U.S. EPA, 1980). Exposure can occur by inhalation, ingestion and skin contact (OSHA/NIOSH, 1980). Dermal exposure to the general population could result from consumer use of dyes packaged for home use, products derived from 3,3'-dimethylbenzidine, as well as products treated with these compounds (U.S. EPA, 1980).

Pertinent data regarding effects of 3,3'-dimethylbenzidine on aquatic biota could not be located in the available literature as cited in the Appendix.

3,3'-Dimethylbenzidine is absorbed after oral or dermal exposure. Rats treated orally with ^{14}C-3,3'-dimethylbenzidine excreted ~40% of the dose of radioactivity in the urine and ~59% in the feces in 8 days (Bowman et al., 1982). Bowman et al. (1982) suggested that biliary excretion was substantial, since ~89% of the fecal radioactivity was present as possible biliary metabolites. Thus, gastrointestinal absorption may be as great as 93%. The appearance of 3,3'-dimethylbenzidine in the urine of occupationally exposed humans indicates that dermal absorption occurs (Meigs et al., 1954). Following absorption, 3,3'-dimethylbenzidine is widely distributed in rats (Bowman et al., 1982). Metabolites identified in the urine of exposed rats were 1,1-diacetyl-3,3'-dimethylbenzidine, alkaline hydrolyzable conjugates and monoacetyl-3,3'-dimethylbenzidine as well as parent compound.

Metabolites identified in the urine of exposed workers were mono- and diacetyl-3,3'-dimethylbenzidine, 5-hydroxy-3,3'-dimethylbenzidine and possibly 3,3'-benzidinedicarboxylic acid.

Griswold et al. (1968) observed an increased incidence of mammary tumors in rats treated by gavage with a total of 500 mg/rat over 30 days and observed for 9 months compared with controls. No tumors were observed in hamsters treated with 3,3'-dimethylbenzidine in the diet at 0.1 or 0.3% for the lifespan (Saffioti et al., 1967; Sellakumar et al., 1969). Ferber (1977) reported a study in which one case of bladder cancer was observed in 1/4 dogs 8 years after being fed 3,3'-dimethylbenzidine at 200 mg/day for 8-9 months. 3,3'-Dimethylbenzidine is being tested by the National Toxicology Program (NTP, 1986). Preliminary pathological results indicate that 3,3'-dimethylbenzidine administered in drinking water induces a carcinogenic effect in F344 rats (Mennear, 1987).

There are numerous positive carcinogenicity studies for 3,3'-dimethylbenzidine as well as some that are negative. Overall the animal evidence is considered sufficient using EPA's weight-of-evidence criteria (Group B2).

Several positive carcinogenicity studies by injection and subcutaneous administration were available as well. In rats given subcutaneous doses of 3,3'-dimethylbenzidine, tumors were observed in the Zymbal gland, preputial gland, forestomach, skin, lung (Pliss and Zabezhinsky, 1970), heart (rhabdomyosarcoma) (Pliss and Vol'fson, 1973) and external auditory canal (Spitz et al., 1950). In rats subcutaneously implanted with pellets containing 3,3'-dimethylbenzidine, Zymbal gland, mammary gland, skin, liver and hematopoietic system tumors were observed (Pliss and Zabezhinsky, 1970). Increased incidences of mammary and lung tumors were observed in the offspring of mice injected subcutaneously during gestation (Golub et al., 1974).

The possible carcinogenic effects of 3,3'-dimethylbenzidine in humans are not assessed adequately to determine the direct effect of 3,3'-dimethylbenzidine alone. Urinary system cancer in workers exposed to mixtures of benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine has been reported (MacAlpine, 1947; Kuratsune and Takemura, 1969); however, adequate interpretion of these results are confounded by the presence of benzidine, a known human bladder carcinogen.

The mutagenicity of 3,3'-dimethylbenzidine has been tested in various prokaryotic and eukaryotic systems. 3,3'-Dimethylbenzidine gave predominantly positive results in frameshift sensitive tester strains of Salmonella typhimurium after liver S-9 metabolic activation (Reid et al., 1984a; Waalkens et al., 1981; Anderson and Styles, 1978; Kenelly et al., 1984; Lazear and Louie, 1977; Prival et al., 1984; Feretti et al., 1977). Positive results were obtained in DNA repair tests in the Escherichia coli growth differential test (Nishioka and Ogasawara, 1978), hepatocytes isolated from rats and hamsters, and in HeLa cells with rat liver metabolic activation (Martin et al., 1978). Positive results were obtained in the sex-linked recessive lethal test using Drosophila melanogaster after feeding adult males 3,3'-dimethylbenzidine (Valencia et al., 1985). Negative results, however, were found in Drosophila for the induction of reciprocal translocations. Positive results were obtained in a micronucleus test in rats (Cihak, 1979), in a testicular DNA synthesis test in mice (Seiler, 1977) and in a cell transformation test in rat embryo cells (Freeman et al., 1973).

The teratogenicity of 3,3'-dimethylbenzidine has not been adequately assessed by a relevant route of administration. Wilson (1955) observed no malformations in progeny of pregnant rats subcutaneously injected with

3,3'-dimethylbenzidine, but did observe a ~7% increase of resorptions. Ema et al. (1984) did not observe fetotoxic effects in progeny of rat dams subcutaneously injected with 3,3'-dimethylbenzidine. Pertinent data regarding the reproductive effects of 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix.

The chronic and subchronic toxicity of 3,3'-dimethylbenzidine has not been studied.

The lack of subchronic and chronic toxicity data on 3,3'-dimethylbenzidine precludes the derivation of an RfD or RQ. 3,3'-Dimethylbenzidine is considered to be an animal carcinogen based on the results of subcutaneous, injection and oral studies. The U.S. EPA (1986a) listed an F factor of 34 $(mg/kg/day)^{-1}$. This F factor would place 3,3'-dimethylbenzidine in Potency Group 2. U.S. EPA (1986a) assigned an EPA weight-of-evidence classification of C; however, this assessment amends that classifiction to Group B2 and a medium hazard ranking under CERCLA. A preliminary q_1^* of 9.2 $(mg/kg/day)^{-1}$ was derived from the Griswold et al. (1968) study. When the current NTP study becomes available, the carcinogenic potency of 3,3'-dimethylbenzidine will be reevaluated.

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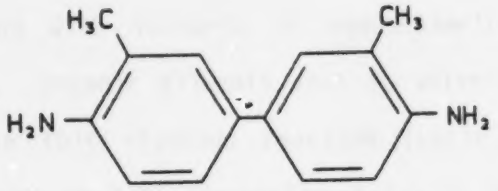
LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
BCF	Bioconcentration factor
CAS	Chemical Abstract Service
DNA	Deoxyribonucleic acid
FMN	Flavin mononucleotide
K_{oc}	Soil sorption coefficient standardized with respect to organic carbon
K_{ow}	Octanol/water partition coefficient
MTD	Maximum tolerated dose
ppb	Parts per billion
RfD	Reference dose
RQ	Reportable quantity
TLV	Threshold limit value
TRNA	Transfer ribonucleic acid
TWA	Time-weighted average
UDS	Unscheduled DNA synthesis
UV	Ultraviolet

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

3,3'-Dimethylbenzidine is also known as o-tolidine, 3,3'-dimethyl-1,1'-biphenyl-4,4'-diamine, diaminoditolyl and C.I. Azoic Diazo Component 113 (C.I. 37230). The structure, molecular weight, empirical formula and CAS Registry number for 3,3'-dimethylbenzidine are as follows:



Molecular weight: 212.28

Empirical formula: $C_{14}H_{16}N_2$

CAS Registry number: 119-93-7

1.2. PHYSICAL AND CHEMICAL PROPERTIES

3,3'-Dimethylbenzidine is a white to reddish crystalline compound at ambient temperature (Windholz, 1983). It is a weak base (IARC, 1972) and its reactions are similiar to those of benzidine, which undergoes chemical reactions characteristic of primary arylamines as well as those reactions related to its difunctional character such as oxidation to quinonoid structures and copolymerization (Ferber, 1978). 3,3'-Dimethylbenzidine forms intense blue compounds with chlorine and bromine vapors (Lurie, 1964). 3,3'-Dimethylbenzidine is soluble in alcohol, ether and dilute acids (Windholz, 1983). Selected physical properties are as follows:

Melting point:	129-131°C	Windholz, 1983
Boiling point:	416-419°C (estimated)	Neely and Blau, 1985
Vapor pressure at 20°C:	2.7×10^{-7} mm Hg (estimated)	Neely and Blau, 1985

Water solubility at 25°C:	2400 mg/ℓ (estimated)	Lyman et al., 1982 (equation 2-2)
Log K_{ow}:	2.34	Hansch and Leo, 1985

1.3. PRODUCTION DATA

3,3'-Dimethylbenzidine is made by the reduction of o-nitrotoluene with zinc dust and caustic soda to hydrazotoluene, which is subsequently rearranged by boiling with sulfuric or hydrochloric acid (Lurie, 1964; Powell et al., 1979). Organic diluents such as solvent naphtha and alcohol are used to moderate this vigorous reaction (Lurie, 1964). No current commercial manufacturers of 3,3'-dimethylbenzidine in the United States were reported (SRI, 1986; USITC, 1985). Table 1-1 lists the U.S. manufacturers or importers of this compound in 1977 and their production volumes.

In 1978, Upjohn discontinued manufacturing this compound and began importing it (Powell et al., 1979). In 1983, 75,307 pounds of 3,3'-dimethylbenzidine was imported into the United States through principal customs districts (USITC, 1984).

1.4. USE DATA

It is estimated that >75% of the 3,3'-dimethylbenzidine consumed is used as an intermediate in the production of dyes and pigments (Powell et al., 1979). Twenty-two azo dyes are produced domestically from 3,3'-dimethylbenzidine (U.S. EPA, 1980), the most important of which are Direct Red 2, Direct Red 39 and Direct Blue 25 (Ferber, 1978). The use pattern for benzidine-based dyes is as follows: paper coloring, 40%; textile coloring, 25%; leather coloring, 15%; diverse applications in the petroleum, rubber plastics, wood, soap, fur and hair dye industries, 20% (NIOSH, 1980).

TABLE 1-1

Manufacturers or Importers of 3,3'-Dimethylbenzidine in 1977*

Company/Location	Manufacturer or Importer	Production/Import Volume (million pounds)
The Upjohn Co. North Haven, CT	manufacturer	confidential
Aceto Chem Co., Inc. Flushing, N.Y.	importer	0.010-0.100
American Hoechst Corp. Bridgewater, NJ	importer	confidential
Nagase America Corp. New York, NY	importer	0.010-0.100
GAF Corp. New York, NY	importer	0.001-0.010

*Source: U.S. EPA, 1977

Approximately 20% of 3,3'-dimethylbenzidine consumed domestically is used in the production of polyurethane-based high strength elastomers, coatings and rigid plastics. 3,3'-Dimethylbenzidine is also used for chlorine detection and other laboratory procedures; however, this accounts for <5% of the 3,3'-dimethylbenzidine used domestically (Powell et al., 1979).

1.5. SUMMARY

3,3'-Dimethylbenzidine is a white to reddish crystalline compound at ambient temperature. It is sparingly soluble in water and is soluble in ether, alcohol and dilute acids (Windholz, 1983). It is a weak base and undergoes reactions similar to those of benzidine (IARC, 1972; Ferber, 1978). 3,3'-Dimethylbenzidine is made by the reaction of o-nitrotoluene with zinc dust and caustic soda to form hydrazotoluene, which is subsequently rearranged by boiling with sulfuric or hydrochloric acid (Lurie, 1964; Powell et al., 1979). No current commercial manufacturers of 3,3'-dimethylbenzidine in the United States were reported (SRI, 1986; USITC, 1985). In 1983, 75,307 pounds of this compound was imported into the United States through principal customs districts (USITC, 1984). It is estimated that >75% of the 3,3'-dimethylbenzidine consumed is used as an intermediate in the production of azo dyes and pigments used for coloring paper, textiles and leathers and for diverse applications in the petroleum, rubber, plastics, wood, soap, fur and hair dye industries (Powell et al., 1979; NIOSH, 1980). Approximately 20% of the 3,3'-dimethylbenzidine consumed domestically is used in the production of polyurethane-based high strength elastomers, coatings and rigid plastics and <5% is used for chlorine detection and other laboratory procedures (Powell et al., 1979).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

3,3'-Dimethylbenzidine is a weak base that may be protonated under acidic conditions to form salts that are more water soluble than the parent compound. Protonation can alter the behavior of a compound in water and soil, for example, by decreasing volatilization as well as increasing or decreasing adsorption to soil, sediments and suspended solids in water.

2.1. WATER

2.1.1. Hydrolysis. Based on the structure of 3,3'-dimethylbenzidine, this compound is likely to be resistant to hydrolysis (Lyman et al., 1982).

2.1.2. Oxidation. Pertinent data regarding the oxidation of this compound could not be located in the available literature as cited in the Appendix; however, unsubstituted benzidine is very rapidly oxidized by Fe(+3) and other naturally occurring cations found in natural waters (U.S. EPA, 1979). Because of the two methyl substituents on the aromatic ring, 3,3'-dimethylbenzidine may have a higher tendency to undergo this oxidation by metal cations. Based on the fate of benzidine in water (U.S. EPA, 1979), oxidation by other oxidants including molecular O_2 and HO_2 radicals will also take place, although oxidation by metal cations in natural water may be more rapid. On the basis of the estimated oxidation half-life of benzidine in natural water (U.S. EPA, 1979), the half-life of 3,3'-dimethylbenzidines is estimated to be <4 hours.

2.1.3. Photolysis. Dimethylbenzidine in methanol strongly absorbs UV light in the environmentally significant wavelength range of >290 nm (Sadtler, n.d.). These data indicate that direct photolytic reactions may be significant in removing 3,3'-dimethylbenzidine from water. Experimental data on the photolysis of this compound could not be located in the available literature as cited in the Appendix.

2.1.4. Other Chemical Reactions. Schmitz and Rooze (1981) reported that 3,3'-dimethylbenzidine reacts rapidly with chlorine and with chlorine dioxide in aqueous solution, suggesting that these reactions could contribute significantly to the removal of 3,3'-dimethylbenzidine from water, particularly during the disinfection of drinking water.

2.1.5. Biodegradation. Pertinent data regarding the biodegradation of 3,3'-dimethylbenzidine were limited. An aerobic biodegradation screening study using 20 mg/ℓ 3,3'-dimethylbenzidine inoculated with activated sludge at 25°C resulted in 99-100% depletion of the parent compound in 6 hours (Baird et al., 1977). The metabolic intermediates, however, were speculated to be toxic to microorganisms and the oxygen consumption in experiments with 3,3'-dimethylbenzidine was less than the endogenous process.

2.1.6. Adsorption. Based on observed covalent binding of primary amines to humates (Parris, 1980) and the estimated K_{oc} of 60-271 (Section 2.3.2.), physical adsorption and covalent bonding to suspended solids and sediments in water may be significant. In fact, based on the studies with benzidine and 3,3'-dichlorobenzidine (U.S. EPA, 1979) it is likely that adsorption by suspended microcrystalline clays may be the most important process and the half-life of that process may be <1 hour.

2.1.7. Volatilization. 3,3'-Dimethylbenzidine should not volatilize significantly from water because of possible protonation in water at pH<7. The adsorption to microcrystalline clays and covalent binding with humates in water will also reduce volatility. The volatility may be higher at pH>7 than at pH<7.

2.1.8. **Bioaccumulation.** The BCF for 3,3'-dimethylbenzidine has been estimated using the following recommended linear regression equations (Lyman et al., 1982):

$$\log BCF = 0.76 \log K_{ow} - 0.23 \quad (2\text{-}1)$$

$$\log BCF = 2.791 - 0.564 \log S(mg/\ell) \quad (2\text{-}2)$$

Based on the estimated log K_{ow} of 2.34 and solubility of 2400 mg/ℓ at 25°C, the BCF for 3,3'-dimethylbenzidine has been calculated to be 35 and 8 from Equations 2-1 and 2-2, respectively. These BCF values indicate that 3,3'-dimethylbenzidine should not bioaccumulate significantly in aquatic organisms.

2.2. AIR

2.2.1. **Reaction with Hydroxyl Radicals.** 3,3'-Dimethylbenzidine in the vapor phase will react in the atmosphere with photochemically generated hydroxyl radicals. The hydroxyl reaction rate constant has been estimated to be 6.02×10^{-11} cm³/molecule-sec at 25°C. Assuming an ambient hydroxyl radical concentration of 8.0×10^5 molecules/cm³, the reaction half-life has been estimated to be 4.0 hours (U.S. EPA, 1986b).

2.2.2. **Reaction with Ozone.** 3,3'-Dimethylbenzidine is not susceptible to oxidation by ozone (U.S. EPA, 1986b).

2.2.3. **Photolysis.** Since 3,3'-dimethylbenzidine in methanol strongly absorbs UV light of wavelengths >290 nm (Sadtler, n.d.), direct photolysis may be significant in the troposphere.

2.3. SOIL

2.3.1. **Chemical and Microbial Degradation.** Pertinent data regarding the chemical or microbial degradation of 3,3'-dimethylbenzidine in soil could not be located in the available literature. Based on the molecular structure of 3,3'-dimethylbenzidine, this compound is likely to be resistant to

hydrolysis (Lyman et al., 1982). Benzidine, a cogener of 3,3'-dimethylbenzidine is reported to undergo rapid oxidation in clay soils with a half-life of <1 day (U.S. EPA, 1980; Lapp et al., 1981). Therefore, it is expected that 3,3'-dimethylbenzidine will also undergo this soil-catalyzed oxidation and the half-life of this reaction may be <1 day.

2.3.2. Adsorption. Parris (1980) experimentally determined that primary amines covalently bind to humates (soil organic matter) initially by a rapid, reversible reaction followed by a slower irreversible binding process. The K_{oc} for 3,3'-dimethylbenzidine has been calculated using the following linear regression equations (Lyman et al., 1982):

$$\log K_{oc} = -0.55 \log S(mg/\ell) + 3.64 \quad (2\text{-}3)$$

$$\log K_{oc} = -0.54 \log S(\text{mol fraction}) + 0.44 \quad (2\text{-}4)$$

Based on a water solubility of 2400 mg/ℓ at 25°C, K_{oc} values of 60 and 271 were calculated from Equations 2-3 and 2-4, respectively. These K_{oc} values suggest that 3,3'-dimethylbenzidine may be moderately mobile in soil (Swann et al., 1983); however, since benzidine and substituted benzidine are known to adsorb strongly and rapidly to microcrystalline clays (U.S. EPA, 1979), 3,3'-dimethylbenzidine may be significantly immobilized in certain soil types by physical adsorption and covalent binding. In acidic soils some mobilization of the compound is expected because of protonation.

2.3.3. Volatilization. Pertinent experimental data regarding volatilization of 3,3'-dimethylbenzidine in soil could not be located in the available literature as cited in the Appendix. Based on the water solubility (2400 mg/ℓ at 25°C), possible protonation in acidic soils and adsorption and covalent binding to soils (see Section 2.3.2.), volatilization from wet and dry soil surfaces is not expected to be significant.

2.4. SUMMARY

3,3'-Dimethylbenzidine is a weak base and as such, may be protonated and form salts that are more soluble than the parent compound. Variations in the behavior of 3,3'-dimethylbenzidine in the environment may result from protonation. If released to water, 3,3'-dimethylbenzidine is expected to be oxidized by Fe(+3) and other cations fairly rapidly (U.S. EPA, 1979). The half-life of this oxidation may be <4 hours. It is likely to be resistant to hydrolysis (Schmitz and Rooze, 1981; Lyman et al., 1982). Potential exists for significant photolysis of 3,3'-dimethylbenzidine. Adsorption to microcrystalline clay and covalent binding to suspended solids and sediments may be significant (U.S. EPA, 1979; Parris, 1980). In fact, adsorption by microcrystalline clays may be the most important process in water. Bioaccumulation in aquatic organisms and volatilization from water should not be significant removal processes. If released to air, vapor phase 3,3'-dimethylbenzidine is predicted to react with hydroxyl radicals and has an estimated half-life of 4.0 hours at 25°C (U.S. EPA, 1986b). Direct photolysis in the troposphere is also likely to be a significant removal mechanism. Reaction with ozone is unlikely (U.S. EPA, 1986b). If released to soil, 3,3'-dimethylbenzidine is likely to be resistant to hydrolysis (Lyman et al., 1982). 3,3'-Dimethylbenzidine may be immobilized in soil by physical adsorption and covalent binding. Some mobilization may occur in acidic soils because of protonation. Volatilization from wet and dry soil surfaces should not be significant. Soil-catalyzed oxidation of 3,3'-dimethylbenzidine may be the most significant reaction of this chemical and the half-life of this reaction may be <1 day (U.S. EPA, 1980; Lapp et al., 1981).

3. EXPOSURE

Monitoring data regarding 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix. This compound is not known to occur naturally in the environment (U.S. EPA, 1980). Potential sources of major release are in airborne particulate matter and wastewater from manufacturing and processing plants for dyes and pigments derived from 3,3'-dimethylbenzidine (U.S. EPA, 1980; Lapp et al., 1981).

Minor sources of release may originate from clothing, colored paper, textiles, leather and other dyed materials that are commonly disposed of in solid waste disposal sites (U.S. EPA, 1980).

The workers who manufacture and handle 3,3'-dimethylbenzidine-based dyes and pigments are likely to be the most exposed segment of the population (U.S. EPA, 1980). Urine samples from some workers exposed to azo dyes during manufacture (two sites) and use (four sites) contained ppb levels of 3,3'-dimethylbenzidine (Lowry et al., 1980). Workers in biochemical and clinical laboratories where 3,3'-dimethylbenzidine is used may also be exposed (U.S. EPA, 1980). Exposure can occur by inhalation, ingestion and skin contact (OSHA/NIOSH, 1980). Dermal exposure to the general population could result from consumer use of dyes packaged for home use, products derived from 3,3'-dimethylbenzidine, as well as products treated with these compounds (U.S. EPA, 1980).

4. PHARMACOKINETICS

4.1. ABSORPTION

Bowman et al. (1982) treated male rats with single oral doses that contained 17 µg of ^{14}C-3,3'-dimethylbenzidine (uniformly ring-labeled) oral 765 µg of unlabeled 3,3-dimethylbenzidine•2HCl. The vehicle was dilute aqueous HCl. After 192 hours, ~40 and 59% of the radioactive dose was excreted in the urine and feces, respectively. As 89% of the fecal radioactivity represented possible biliary metabolites, biliary excretion may have accounted for the high degree of fecal excretion. Therefore, absorption by the gastrointestinal tract may have been at least 93%. Bowman et al. (1982) did not investigate biliary excretion in bile duct cannulated rats, however. Meigs et al. (1954) detected 3,3'-dimethylbenzidine in the urine of occupationally exposed workers, indicating that dermal exposure of humans results in absorption by this route.

4.2. DISTRIBUTION

Bowman et al. (1982) gave rats single oral doses of ^{14}C-3,3'-dimethylbenzidine (see Section 4.1.) and assayed the tissues for distribution 72 hours later. Radioactivity was widely distributed to tissues. The highest levels of ^{14}C were observed in the liver (0.907 µg equiv/g), lung (0.459 µg equiv/g) and kidney (0.130 µg equiv/g). Small amounts of radioactivity (0.004-0.048 µg equiv/g) were also found in brain, muscle, testes, heart, spleen, blood and urinary bladder. Levels in adipose tissue were below detection levels (<0.014 µg equiv/g).

Pliss and Zabezhinsky (1970) subcutaneously injected rats with 3,3'-dimethylbenzidine in sunflower oil at a dose of 20 mg/rat once weekly for 8 months. At 3 days after the last dose, the rats were killed and the levels

of amines were measured for several organs. The highest amine levels (free and bound added together) as mg/g tissue in descending order were observed in the Zymbal gland, kidney, omentum, spleen and liver.

4.3. METABOLISM

Dieteren (1966) identified the urinary metabolites of workers occupationally exposed to 3,3'-dimethylbenzidine. The parent compound was found as well as the metabolites diacetyl-3,3'-dimethylbenzidine, 5-hydroxy-3,3'-dimethylbenzidine or its ester and possibly monoacetyl-3,3'-dimethylbenzidine. The authors emphasized that other metabolites were present as well (e.g., 3,3'-benzidinedicarboxylic acid, which can be obtained by oxidation of the methyl groups).

Bowman et al. (1982) gave Fischer 344 rats single oral doses (see Section 4.1.) of ^{14}C-3,3'-dimethylbenzidine and identified the following urinary metabolites: diacetyl-3,3'-dimethylbenzidine (2.1% of the dose); alkaline hydrolyzable conjugates (0.4%); monoacetyl-3,3'-dimethylbenzidine (0.2%). Other unidentified metabolites were present as well. Unchanged 3,3'-dimethylbenzidine constituted 0.4% of the dose. The interval of peak excretion of metabolites was between 8 and 16 hours.

4.4. EXCRETION

Bowman et al. (1982) gave Fischer 344 rats single oral doses of ^{14}C-3,3'-dimethylbenzidine (see Section 4.1.) and found that ~40 and 59% of the dose of radioactivity was excreted in the urine and feces, respectively, within 192 hours. About 1-6% of the dose was excreted as the parent compound. Urinary and fecal excretion peaked at 16 hours.

4.5. SUMMARY

3,3'-Dimethylbenzidine is absorbed after oral or dermal exposure. Rats treated orally with ^{14}C-3,3'-dimethylbenzidine excreted ~40% of the dose of radioactivity in the urine and ~59% in the feces in 8 days (Bowman et al., 1982). Bowman et al. (1982) suggested that biliary excretion was substantial, since ~89% of the fecal radioactivity was present as possible biliary metabolites. Thus, gastrointestinal absorption may be as great as 93%. The appearance of 3,3'-dimethylbenzidine in the urine of occupationally exposed humans indicates that dermal adsorption occurs (Meigs et al., 1954). Following absorption, 3,3'-dimethylbenzidine is widely distributed in rats (Bowman et al., 1982). Metabolites identified in the urine of exposed rats were diacetyl-3,3'-dimethylbenzidine, alkaline hydrolyzable conjugates and monoacetyl-3,3'-dimethylbenzidine as well as parent compound. Metabolites identified in the urine of exposed workers were mono- and diacetyl-3,3'-dimethylbenzidine, 5-hydroxy-3,3'-dimethylbenzidine and possibly 3,3'-benzidinedicarboxylic acid.

5. EFFECTS

5.1. CARCINOGENICITY

5.1.1. Animal Studies. Griswold et al. (1968) treated a group of twenty 45-day-old female Sprague-Dawley rats by gavage with 10 doses of 50 mg/rat (total 500 mg/rat) 3,3'-dimethylbenzidine in sesame oil at 3-day intervals for 30 days, followed by a 9-month observation period (Table 5-1). Sixteen rats survived the observation period. Rats that died or were killed at the end of the study were necropsied. Histological examination was performed on grossly apparent lesions, mammary glands, intestinal tract, pituitary, liver, ovaries and adrenals. The mammary tumor incidence in treated female rats was 3/16; whereas an incidence of 5/132 was observed in the control data pooled from several experiments. IARC (1972) considered this experiment to be of doubtful significance because of the small number of rats tested.

Saffiotti et al. (1967) gave groups of 30 male and 30 female 9-week-old Syrian golden hamsters 0.1% 3,3'-dimethylbenzidine in the diet for their lifespan. The authors estimated 60 mg of the test substance was ingested per week based on food intake data. Controls were given food that did not contain 3,3'-dimethylbenzidine. No tumors were observed in the treated hamsters. In a similar experiment, Sellakumar et al. (1969) reported negative results in 30 male and 30 female hamsters maintained on a diet containing 0.3% 3,3'-dimethylbenzidine for the lifespan.

Ferber (1977) reported a study in which four young mongrel female dogs were given 200 mg 3,3'-dimethylbenzidine in capsules daily for 8-9 months for a total dose of 50 g/dog. Cystoscopic examinations before and after treatment were negative. One of the four treated dogs developed bladder cancer (papillary tumor and cystitis) 8 years later. The remaining dogs either died of natural causes or were killed, but no tumors were observed.

TABLE 5-1

Mammary Tumor Incidence in 45-Day-Old Female Sprague-Dawley Rats Given 50 mg of 3,3'-Dimethylbenzidine of Unspecified Purity in Sesame Oil at 3-day Intervals for 30 Days, Followed by a 9-Month Observation Period*

Dose	Incidence
0	5/132 (~4%)
50 mg/rat (500 mg total dose)	3/16 (~19%) (NS)

QUALITY OF EVIDENCE

Strength of Study:	A relevant route of administration was used. The MTD was established by pre-testing.
Weakness of Study:	Only one sex of one species was tested. Only one dose was tested and the duration of exposure was not for the lifespan of the organism. A limited number of organs were examined histologically and the data were not statistically analyzed.
Overall Adequacy:	Valid for weight-of-evidence, limited for statistical analysis

*Source: Griswold et al., 1968

3,3'-Dimethylbenzidine is being tested for carcinogenicity by the National Toxicology Program (NTP, 1986). Preliminary results indicate that 3,3'-dimethylbenzidine induces a carcinogenic effect in F344 rats when the compound is administered in drinking water (Mennear, 1987).

Several studies that used less relevant routes of administration were located in the available literature. Pliss and Zabezhinsky (1970) subcutaneously injected 27 male and 26 female rats with 20 mg/rat 3,3'-dimethylbenzidine in sunflower oil once weekly for 13 months and observed tumors in 60% of the treated rats. The first tumor appeared at 8 months, at which time 25 rats/sex survived. Zymbal gland tumors (14/25 males, 6/25 females) were the primary tumor type observed, but tumors also developed in the preputial gland (2/25 males, 1/25 females), forestomach (2/25 males, 0/25 females), skin (2/25 males, 1/25 females) and mammary gland (5/25 females). No treatment-related tumors were observed in a group of 50 control rats injected subcutaneously with sunflower oil (Pliss, 1963). Pliss and Zabezhinsky (1970) also subcutaneously implanted pellets containing 20 mg 3,3'-dimethylbenzidine in 24 male and 24 female rats, once weekly for 14 months. The first tumor appeared at 12 months at which time 16 males and 20 females were alive. The treated rats developed primarily Zymbal gland (6/16 males, 5/20 females) and mammary gland tumors (7/20 females, 0/16 males). Tumors of the skin, liver and hematopoietic system were observed as well. Pliss and Vol'fson (1973) observed a rhabdomyosarcoma of the heart in rats subcutaneously injected, once weekly with 10 mg 3,3'-dimethylbenzidine for 33 weeks. Holland et al. (1974) reported that 18/21 rats given a cumulative subcutaneous dose of 5.4 g/kg over 241 days developed tumors.

Spitz et al. (1950) observed no cirrhosis of the liver or hepatomas in 105 rats subcutaneously injected with 60 mg 3,3'-dimethylbenzidine once weekly for a total dose of 5.5 g. Cancer of the external auditory canal was observed in 4.3% of the treated rats.

Golub et al. (1974) subcutaneously injected BALB/c mice with 2 mg/injection of 3,3'-dimethylbenzidine in sunflower oil 4 or 5 times during the last week of pregnancy. The control group consisted of the progeny of untreated dams. No effects of treatment were reported in the dams. Survival of the progeny of treated dams was diminished; however, no additional data were provided. The progeny of treated dams had a higher total tumor incidence (13/24, 50%) compared with controls (6/30, 20%). Major types of tumors observed in treated mice were lung and mammary tumors.

5.1.2. Human. MacAlpine (1947) reported two cases of urinary tract tumors, which eventually resulted in death of workers occupationally exposed to benzidine and 3,3'-dimethylbenzidine. Kuratsune and Takemura (1969) reported 23 cases of urinary system cancer in Japanese workers exposed to benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine. Fourteen of these workers were exposed for <6 years. The workers exposed in these cases were also exposed to benzidine, a known bladder carcinogen. A review of the carcinogenic effects observed in workers of the dyestuff industry can be found in several publications (NIOSH, 1978, 1980; OSHA/NIOSH, 1980). The available epidemiologic data clearly show that benzidine base dyes are human carcinogens. The data also show that benzidine in combination with 3,3'-dimethyl- and dimethoxybenzidine is carcinogenic to humans. There is, however, no data available for dimethylbenzidine alone.

5.2. MUTAGENICITY

The ability of 3,3'-dimethylbenzidine to cause point mutations in the Salmonella typhimurium histidine reverse mutation test is well established (Table 5-2). In general, 3,3'-dimethylbenzidine was mutagenic in frame-shift-sensitive tester strains (namely, TA98 and TA1538) in the presence of rat, mouse or hamster liver S9 metabolic activation (Reid et al., 1984a,b; Waalkens et al., 1981; Kennelly et al., 1984; Lazear and Louie, 1977; Prival et al., 1984; Anderson and Styles, 1978; Ferretti et al., 1977). Negative or weakly positive results were obtained in base-pair substitution tester strains (i.e., TA1535, TA1537, TA100) of Salmonella.

Nishioka and Ogasawara (1978) obtained positive results in a growth differential test of Escherichia coli using DNA repair-deficient and proficient strains. Kornbrust and Barfknecht (1984, 1985) observed positive results in DNA repair tests using hepatocytes isolated from both male Sprague-Dawley rats or male Golden Syrian hamsters. 3,3'-Dimethylbenzidine induced unscheduled DNA synthesis in HeLa cells with rat liver metabolic activation (Martin et al., 1978). In the sex-linked recessive lethal test in Drosophila melanogaster, positive results were obtained when 3,3'-dimethylbenzidine was administered by feeding adult males and produced equivocal results after adult injection (Valencia et al., 1985). However, 3,3'-dimethylbenzidine did not induce reciprocal translocations in Drosophila (Valencia et al., 1985). A statistically significant treatment-related increased frequency of micronucleated polychromatic erythrocytes was observed in bone marrow smears from Wistar rats given 3,3'-dimethylbenzidine in olive oil by stomach tube (Cihak, 1979). Testicular DNA synthesis was inhibited in mice given 3,3'-dimethylbenzidine orally (Seiler, 1977). Freeman et al. (1973) obtained positive results in in vitro transformation tests using Fischer rat embryo cell line F1706 P88.

TABLE 5-2

Mutagenicity Testing of 3,3'-Dimethylbenzidine

Assay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA100, TA98, TA1538, TA1535	3,3'-dimethyl-benzidine dihydrochloride/ >98% pure	plate incorporation	100-500 µg/plate (expressed as actual amount of free base)	+ rat S-9	+/-	Positive dose-related response in strains TA98 and TA1538; negative results for strains TA100 and TA1535.	Reid et al., 1984a
Reverse muation	S. typhimurium TA98, TA1538	3,3'-dimethyl-benzidine/ >99% pure	plate incorporation	62.5-1000 µg/plate	± rat S-9	±	Positive dose-related response in both strains in the presence of S9 activation	Waalkens et al., 1981
Reverse mutation	S. typhimurium TA100, TA1538, TA1537	3,3'-dimethyl-benzidine/NR	plate incorporation	62.5-1000 µg/plate	± rat S-9	- -	NC	Waalkens et al., 1981
Reverse mutation	S. typhimurium TA1538,TA100	3,3'-dimethyl-benzidine/NR	plate incorporation	4-2500 µg/plate	+ rat S-9	+	NC	Anderson and Styles, 1978
Reverse mutation	S. typhimurium TA1535, TA98	3,3'-dimethyl-benzidine/NR	plate incorporation	4-2500 µg/plate	+ rat S-9	-	NC	Anderson and Styles, 1978
Reverse mutation	S. typhimurium TA98, TA100	3,3'-dimethyl-benzidine/NR	plate incorporation	50-100 µg/plate	+ mouse liver S-9	+	Weak dose-related response	Lazear and Loule, 1977
Reverse mutation	S. typhimurium TA1538	3,3'-dimethyl-benzidine/NR	plate incorporation	100 µg/plate	± rat S-9	±	NC	Ferretti et al., 1977
Reverse mutation	S. typhimurium TA98, TA1538	3,3'-dimethyl-benzidine/NR	plate incorporation	10-100 µg/plate	+ rat S-9	+	Dose-related increase in both strains	Kenelly et al., 1984
Reverse mutation	S. typhimurium TA1538	3,3'-dimethyl-benzidine dihydrochloride/98% pure	preincubation	0-1.0 µmoles/assay[a]	+ rat S-9	+	Reduction system[b] used was an extract of rat cecal flora	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	3,3'-dimethyl-benzidine dihydrochloride/98% pure	preincubation	0-1.0 µmoles/assay[a]	+ rat S-9	+	No reduction system[b] was used	Reid et al., 1984b

TABLE 5-2 (cont.)

Assay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	S. typhimurium TA1538	3,3'-dimethyl-benzidine dihydrochloride/98% pure	plate incorporation	0-1.0 µmoles/assay[a]	+ hamster S-9	+	Reduction system[b] used was hamster S-9 with FMN supplementation.	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	3,3'-dimethyl-benzidine dihydrochloride/98% pure	plate incorporation	0-1.0 µmoles/assay[a]	+ hamster S-9	+	Reduction system[b] was used; treatment-related increase in His^+ revertants	Reid et al., 1984b
Reverse mutation	S. typhimurium TA1538	3,3'-dimethyl-benzidine dihydrochloride/98% pure	plate incorporation	0-1.0 µmoles/assay[a]	+ hamster S-9	+	Reduction system[b] used was rat cecal flora; positive dose-related increase in His^+ revertants	Reid et al., 1984b
Reverse mutation	S. typhimurium TA98	3,3'-dimethyl-benzidine/NR	plate incorporation	0-1.0 µmoles/plate	+ hamster S-9 ± FMN	+ +	Dose-related increase in His^+ revertants for both strains with or without FMN supplementation	Prival et al., 1984
DNA repair	Escherichia coli SP2, WP2 uvrA, CM571, WP100	3,3'-dimethyl-benzidine/NR	liquid and/or solid	NR	NR	+	NC	Nishioka and Ogasawara, 1978
DNA repair	hepatocytes from male Sprague-Dawley rats or male Syrian Golden hamsters	3,3'-dimethyl-benzidine/85%	coverslips	10^{-4} to 10^{-6} M	NA	+	Similar results were obtained for both species	Kornbrust and Barfknecht, 1984, 1985
UDS	HeLa cells	3,3'-dimethyl-benzidine/NR	liquid suspension	10^{-4} to 10^{-6} M	+ rat S-9	+	NC	Martin et al., 1978
Sex-linked recessive lethal	Drosophila melanogaster	3,3'-dimethyl-benzidine dihydrochloride/NR	feeding	14,000 ppm	NA	+	NC	Valencia et al., 1985

TABLE 5-2 (cont.)

Assay	Indicator/ Organism	Compound/ Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Sex-linked recessive lethal	D. melanogaster	3,3'-dimethyl-benzidine dihydrochlo-ride/NR	injection	2750 ppm	NA	?	NC	Valencia et al., 1985
Reciprocal translocation	D. melanogaster	3,3'-dimethyl-benzidine dihydrochlo-ride/NR	feeding	14,000 ppm	NA	-	NC	Valencia et al., 1985
Micronucleus test	bone marrow of Wistar rats	3,3'-dimethyl-benzidine/NR	stomach tube	50-200 mg/kg 30 and 6 hours before prep-aration of bone marrow smears	none	+	Dose-related effect	Cihak, 1979
Inhibition of testicular DNA synthesis	mice	3,3'-dimethyl-benzidine/NR	oral	100 mg/kg	none	+	$p<0.05$	Seller, 1977
In vitro transformation	Fischer rat embryo cells	3,3'-dimethyl-benzidine/NR	cell cultures	1.0-100 µg/mℓ	none	+	A negative result was obtained at 1.0 µg/mℓ; positive results ob-tained for concentra-tions ranging from 5-50 µg/mℓ; 100 µg/mℓ ranging from 5-50 µg/mℓ; 100 µg/mℓ was toxic to the cells	Freeman et al., 1973

[a]Dose/assay = the amount of 3,3'-dimethylbenzidine added: (1) overnight incubation mixture for the cecal bacterial reduction system (see below) and (2) to the S-9 mix for the FMN system (see below).

[b]The dye was sequentially reduced and oxidized by rat cecal flora or hamster S-9 supplemented with FMN before rat or hamster S-9 activation.

NR = Not reported; NC = no comment; NA = not applicable

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5.3. TERATOGENICITY

Pertinent data regarding the teratogenicity of 3,3'-dimethylbenzidine administered by the oral or inhalation route could not be located in the available literature as cited in the Appendix. Wilson (1955) observed no malformations in progeny of rats subcutaneously injected with 30 mg of 3,3'-dimethylbenzidine on days 7, 8 and 9 of gestation. The incidence of resorptions was ~7%. No control data or maternal toxicity data were reported. Ema et al. (1984) observed no fetotoxicity in progeny of Wistar rat dams subcutaneously injected with 3,3'-dimethylbenzidine on day 7 of pregnancy.

5.4. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding other reproductive effects of 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix.

5.5. CHRONIC TOXICITY

Pertinent data regarding the oral or inhalation chronic or subchronic toxicity of 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix.

5.6. OTHER RELEVANT INFORMATION

Soloimskaya (1970) subcutaneously injected rats with 100 mg/kg 3,3'-dimethylbenzidine and observed increased monoamine oxidase and histaminase activity. In rats subcutaneously injected with 100 mg/kg once weekly for 2 months, increased activity of the enzymes catalyzing oxidative deamination was observed.

Emmett et al. (1985) demonstrated by changes in crossed immunoelectrophoresis patterns that 3,3'-dimethylbenzidine binds to serum proteins. As demonstrated by autoradiography, 3,3'-dimethylbenzidine bound to α and β

lipoproteins. The authors suggested that bound 3,3'-dimethylbenzidine may be transported to tissues by serum proteins. Wise et al. (1984) reported that dog bladder transitional epithelial prostaglandin H synthase activated 3,3'-dimethylbenzidine binding to protein, tRNA and DNA. Tsurata et al. (1985) reported that the peroxidase/H_2O_2 system catalyzed the binding of 3,3'-dimethylbenzidine to DNA.

5.7. SUMMARY

The carcinogenicity of 3,3'-dimethylbenzidine has not been adequately assessed by oral and inhalation routes of administration. However, there is a wealth of animal data showing that a high level and varied carcinogenic responses do occur in tissues exposed to 3,3'-dimethylbenzidine. Griswold et al. (1968) observed an increased incidence of mammary tumors in rats treated by gavage with a total of 500 mg/rat over 30 days and observed for 9 months compared with controls. IARC (1972), however, considered this experiment to be of doubtful significance because of the small sample size (20 rats).

No tumors were observed in hamsters treated with 3,3'-dimethylbenzidine in the diet at 0.1 or 0.3% for the lifespan (Saffioti et al., 1967; Sellakumar et al., 1969). Ferber (1977) reported a study in which one case of bladder cancer was observed in 1/4 dogs 8 years after being fed 3,3'-dimethylbenzidine at 200 mg/day for 8-9 months. 3,3'-Dimethylbenzidine has been tested by the National Toxicology Program (NTP, 1986). Preliminary results of histopathology indicate that 3,3'-dimethylbenzidine induces a carcinogenic effect in F344 rats when the compound is administered in drinking water (Mennear, 1987).

Numerous carcinogenicity studies by a less relevant route of administration were available as well. In rats given subcutaneous doses of 3,3'-dimethylbenzidine, tumors were observed in the Zymbal gland, preputial gland, forestomach, skin, lung (Pliss and Zabezhinsky, 1970), heart (rhabdomyosarcoma) (Pliss and Vol'fson 1973), and external auditory canal (Spitz et al., 1950). In rats subcutaneously implanted with pellets containing 3,3'-dimethylbenzidine, Zymbal gland, mammary gland, skin, liver and hematopoietic system tumors were observed (Pliss and Zabezhinsky, 1970).

Increased incidences of mammary and lung tumors were observed in the offspring of mice injected subcutaneously during gestation (Golub et al., 1974). The possible carcinogenic effect of 3,3'-dimethylbenzidine in humans has not been assessed adequately. Urinary system cancer in workers exposed to mixtures of benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine has been reported (MacAlpine, 1947; Kuratsune and Takemura, 1969); however, adequate interpretion of these results are confounded by the presence of benzidine, a known human bladder carcinogen.

The mutagenicity of 3,3'-dimethylbenzidine has been tested in various prokaryotic and eukaryotic systems. 3,3'-Dimethylbenzidine gave predominantly positive results in the S. typhimurium reverse mutation test with S-9 metabolic activation (Reid et al., 1984a; Waalkens et al., 1981; Anderson and Styles, 1978; Kenelly et al., 1984; Lazear and Louie, 1977; Prival et al., 1984; Feretti et al., 1977). Positive results were obtained in DNA repair tests in Escherichia coli (Nishioka and Ogasawara, 1978) and in hepatocytes isolated from rats and hamsters. 3,3'-Dimethylbenzidine induced UDS in HeLa cells with metabolic activation (Martin et al., 1978). Positive and negative results, respectively, were obtained in oral and injection studies in the sex-linked recessive lethal tests in Drosophila melanogaster

(Valencia et al., 1985). Positive results were obtained in a micronucleus test in rats (Cihak, 1979), in a testicular DNA synthesis test in mice (Seiler, 1977) and in a cell transformation test in rat embryo cells (Freeman et al., 1973).

The teratogenicity of 3,3'-dimethylbenzidine has not been adequately assessed by a relevant route of administration. Wilson (1955) observed no malformations in progeny of pregnant rats subcutaneously injected with 3,3'-dimethylbenzidine, but did observe an ~7% increase of resorptions. Ema et al. (1984) did not observe fetotoxic effects in progeny of rat dams subcutaneously injected with 3,3'-dimethylbenzidine. Pertinent data regarding the reproductive effects of 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix.

The chronic and subchronic toxicity of 3,3'-dimethylbenzidine has not been studied.

6. AQUATIC TOXICITY

Pertinent data regarding effects of 3,3'-dimethylbenzidine on aquatic biota could not be located in the available literature as cited in the Appendix.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

ACGIH (1985-1986, 1986) did not recommend or adopt a TLV-TWA for 3,3'-dimethylbenzidine and considered this compound to be a suspected human carcinogen. U.S. EPA (1981) decided not to require additional health and environmental effects testing of benzidine, o-tolidine and o-dianisidine based dyes. NIOSH (1978) recommended that 3,3'-dimethylbenzidine "be handled as a suspect human carcinogen," and that "exposure to o-tolidine be kept as low as possible through strict adherence to a program of monitoring, engineering controls and stringent work practices." Occupational exposure should not exceed 20 $\mu g/m^3$ in air, "determined from an air sample collected at 0.2 liters/minute for 60 minutes." Users of laboratory test tapes and kits containing 3,3'-dimethylbenzidine were excluded from monitoring and surveillance requirements of this recommended standard. OSHA/NIOSH (1980) published a Health Hazard Alert for 3,3'-dimethylbenzidine-based dyes.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic biota from the effects of 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

The carcinogenicity of 3,3'-dimethylbenzidine has not been adequately assessed by the oral and inhalation routes of administration. However, there is a wealth of animal data showing that a high level and varied carcinogenic responses do occur in tissues exposed to 3,3'-dimethylbenzidine. Griswold et al. (1968) observed a 19% incidence of mammary tumors in rats treated by gavage with a total of 500 mg/rat over 30 days and observed for 9 months compared with 4% in control rats, which were pooled from several experiments. IARC (1972), however, considered this experiment to be of doubtful significance because of the small sample size (20 rats).

No tumors were observed in hamsters treated with 3,3'-dimethylbenzidine in the diet at 0.1 or 0.3% for the lifespan (Saffioti et al., 1967; Sellakumar et al., 1969). Ferber (1977) reported a study in which one case of bladder cancer was observed in 1/4 dogs 8 years after being fed 3,3'-dimethylbenzidine at 200 mg/day for 8-9 months. 3,3'-Dimethylbenzidine has been tested by the National Toxicology Program (NTP, 1986). Preliminary histopathology data indicate that 3,3'-dimethylbenzidine induces a carcinogenic effect in F344 rats when the compound is administered in drinking water (Mennear, 1987).

Numerous carcinogenicity studies by subcutaneous and injection administration are available as well. In rats given subcutaneous doses of 3,3'-dimethylbenzidine, tumors were observed in the Zymbal gland, preputial gland, forestomach, skin, lung (Pliss and Zabezhinsky, 1970), heart (rhabdomyosarcoma) (Pliss and Vol'fson 1973), and external auditory canal (Spitz et al., 1950). In rats subcutaneously implanted with pellets containing 3,3'-dimethylbenzidine, Zymbal gland, mammary gland, skin, liver and hematopoietic system tumors were observed (Pliss and Zabezhinsky, 1970).

Increased incidences of mammary and lung tumors were observed in the offspring of mice injected subcutaneously during gestation (Golub et al., 1974). The possible carcinogenic effect of 3,3'-dimethylbenzidine in humans has not been assessed adequately. Urinary system cancer in workers exposed to mixtures of benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine has been reported (MacAlpine, 1947; Kuratsune and Takemura, 1969); however, adequate interpretion of these results are confounded by the presence of benzidine, a known human bladder carcinogen. Given the available positive response data showing that 3,3'-dimethylbenzidine causes a carcinogenic response in various animal tissues following tissue exposure, and knowledge that 3,3'-dimethylbenzidine has been identified in humans exposed to benzidine based dyes, 3,3'-dimethylbenzidine is given an EPA Group B2 weight-of-evidence ranking. Preliminary NTP results of a new bioassay of 3,3'-dimethylbenzidine in drinking water are supportive of this as well as a recognition that, while information for 3,3'-dimethylbenzidine alone is nonexistent, the information for 3,3'-dimethylbenzidine and benzidine and 3,3'-dimethoxybenzidine are clearly positive for humans.

The mutagenicity of 3,3'-dimethylbenzidine has been tested in various prokaryotic and eukaryotic systems. 3,3'-Dimethylbenzidine has been shown to produce gene mutations in Salmonella typhimurium after liver S-9 metabolic activation (Reid et al., 1984a; Waalkens et al., 1981; Anderson and Styles, 1978; Kenelly et al., 1984; Lazear and Lowie, 1977; Feretti et al., 1977) and in Drosophila melanogaster after feeding adult males (Valencia et al., 1985). Positive results were obtained in DNA repair tests in Escherichia coli (Nishioka and Ogasawara, 1978), in hepatocytes isolated from rats and hamsters, and in HeLa cells with rat liver metabolic activation (Martin et al., 1978). Positive results were obtained in a micronucleus test in rats (Cihak, 1979), but negative results were reported for

reciprocal translocations in Drosophila (Valencia et al., 1985). In a testicular DNA synthesis test in mice positive results were found (Seiler, 1977). 3,3'-Dimethylbenzidine was reported to be positive in a cell transformation test in rat embryo cells (Freeman et al., 1973).

Therefore, the weight-of-evidence indicates that 3,3'-dimethylbenzidine is mutagenic and may pose a risk to somatic cells. Mutations in somatic cells may lead to the onset of cancer, developmental abnormalities, and possibly other diseases. Further, 3,3'-dimethylbenzidine induces heritable mutations in Drosophila and affects testicular DNA synthesis in mice. These findings suggest that 3,3'-dimethylbenzidine (or an active form) may reach the mammalian gonad and produce mutations in germ cells. Germ cell mutations may be passed on to future generations and increase the incidence of genetic disease in the population. The above conclusions are consistent with the weight-of-evidence approach described in the U.S. EPA guidelines for mutagenicity risk assessment (U.S. EPA, 1986c).

The teratogenicity of 3,3'-dimethylbenzidine has not been adequately assessed by a relevant route of administration. Wilson (1955) observed no malformations in progeny of pregnant rats subcutaneously injected with 3,3'-dimethylbenzidine, but did observe an ~7% increase of resorptions. Ema et al. (1984) did not observe fetotoxic effects in progeny of rat dams subcutaneously injected with 3,3'-dimethylbenzidine. Pertinent data regarding the reproductive effects of 3,3'-dimethylbenzidine could not be located in the available literature as cited in the Appendix.

The chronic toxicity of 3,3'-dimethylbenzidine has not been studied.

3,3'-Dimethylbenzidine is considered to be an animal carcinogen and is suspected of being carcinogenic to humans (IARC, 1972, 1979; NIOSH, 1978; OSHA/NIOSH, 1980; ACGIH, 1986) and by EPA (with this assessment). An EPA 1986 (reportable quantity) carcinogenicity evaluation, which assigned a

weight of evidence of limited animal data (Group C), is superceded by this Group B2 ranking in this risk assessment. NIOSH (1978) recommended that 3,3'-dimethylbenzidine be handled as a suspect human carcinogen based upon positive results in mutagenicity studies and development of tumors in rodents. OSHA/NIOSH (1980) published a Health Hazard Alert for 3,3'-dimethylbenzidine based dyes. ACGIH (1985-1986, 1986) considered 3,3'-dimethylbenzidine a suspected human carcinogen and noted its structural similarity to the known human bladder carcinogen, benzidine.

Given the above carcinogenicity classifications, it is inappropriate to derive an RfD (formerly called ADI). For carcinogenicity, however, consideration of deriving a cancer potency is appropriate. All of the studies that used a relevant route of administration (oral) are flawed to varying degrees and thus the quantitative risk assessment will necessarily have some additional uncertainty.

Griswold et al. (1968) observed an increased incidence of mammary tumors (3/16, 19%) in 20 female rats given 10 doses of 50 mg of 3,3'-dimethylbenzidine by gastric intubation over 30 days, followed by a 9-month observation period compared with pooled controls (5/132, 4%). The purity of the compound administered was not reported, only one sex was tested for a period of time (30 days), which was substantially less than the lifespan of the animal (2 years), size of the group tested was small (20) and was compared with pooled controls, and limited histopathological examination was performed. The data were also not statistically analyzed by the author. IARC (1972) considered this study to be of doubtful significance because of the small sample size tested. While the noted deficiencies exist, this study has been selected for derivation of cancer potency. The q_1^* was determined from this data to be 9.2 $(mg/kg/day)^{-1}$ as shown in Table 8-1.

TABLE 8-1

Derivation of Potency Factor (F factor) for 3,3'-Dimethylbenzidine

Reference:	Griswold et al., 1968	
Exposure route:	gastric intubation with sesame oil	
Species:	rat	
Strain:	Sprague-Dawley	
Sex:	female	
Body weight:	0.35 kg (assumed)	
Duration of treatment:	1 month (10 doses 3 days apart)	
Duration of study:	9 months	
Lifespan of animal:	24 months (assumed)	
Tumor site, type:	mammary carcinomas and hyperplasia	
	Control	Animal
Nominal dose:	0.00	500.00 mg (total lifetime dose)
Average daily dose:	0.00	5.29 mg/kg/day
Equivalent human dose:	0.00	0.90 mg/kg/day (surface-area adj)
Tumor incidence:	5/132	3/16
Potency (q_1*):	9.2 $(mg/kg/day)^{-1}$	
Potency ($1/ED_{10}$):	34.1	

Saffioti et al. (1967) and Sellakumar et al. (1969) gave hamsters 0.1 and 0.3% dietary 3,3'-dimethylbenzidine for their lifespan and observed no tumors. Ferber (1977) observed bladder cancer 1/4 dogs 8 years after being fed a total of 50 g 3,3'-dimethylbenzidine for 8-9 months. This study was not reported in sufficient detail to support a risk assessment. Numerous studies in which 3,3-dimethylbenzidine was administered subcutaneously were available; however, a conventional risk assessment cannot be based on these studies because of the routes of administration and the related difficulty of estimating an effective dose comensurate with oral or inhalation exposure. Urinary system cancer in workers exposed to mixtures of benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine has been reported (MaCalpine, 1947; Kuratsune and Takemura, 1969); however, adequate interpretation of these results for 3,3'-dimethylbenzidine are confounded by the presence of benzidine, a known human bladder carcinogen. NTP (1986) is testing 3,3'-dimethylbenzidine for carcinogenicity. Preliminary results of histopathology indicate that 3,3'-dimethylbenzidine induces a carcinogenic effect in F344 rats when the compound is administered in drinking water (Mennear, 1987).

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

No chronic or subchronic oral or inhalation studies of 3,3'-dimethylbenzidine were available; therefore, data are insufficient to derive an RQ based on chronic toxicity (Table 9-1).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

3,3'-Dimethylbenzidine is considered to be an animal and/or suspected human carcinogen [NIOSH, 1978; OSHA/NIOSH, 1980; ACGIH, 1985-1986, 1986; IARC, 1972, 1979; and EPA (in this assessment)]; however, most oral studies were sufficiently flawed and precluded their use in quantitative risk assessment. In female rats given 10 doses of 50 mg 3,3'-dimethylbenzidine by gastric intubation over 30 days followed by a 9-month observation period, Griswold et al. (1968) observed an increased incidence of mammary tumors (3/16, 19%) compared with pooled controls (5/132, 4%). The purity of the compound administered was not reported, only one sex was tested for a period of time (30 days), which was substantially less than the lifespan of the animal (2 years), size of the group tested was small (20) and was compared with pooled controls, and limited histopathological examination was performed. The data were also not statistically analyzed. IARC (1972) considered this study to be of doubtful significance because of the small sample tested. Saffioti et al. (1967) and Sellakumar et al. (1969) gave hamsters 0.1 and 0.3% dietary 3,3'-dimethylbenzidine for their lifespan and observed no tumors. Ferber (1977) observed bladder cancer in one of four dogs 8 years after being fed a total of 50 g 3,3'-dimethylbenzidine for 8-9 months. This study was not reported in sufficient detail to support a risk assessment. Numerous studies in which 3,3'-dimethylbenzidine was administered subcutaneously were available (see Section 5.1.); however, they cannot support a risk assessment because of the irrelevant route of administration.

TABLE 9-1

3,3'-Dimethylbenzidine

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	
Dose:	
Effect:	
Reference:	
RV_d:	
RV_e:	
Composite Score:	
RQ:	Data are not sufficient for deriving an RQ

Urinary system cancer in workers exposed to mixtures of benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine has been reported (MacAlpine, 1947; Kuratsune and Takemura, 1969); however, adequate interpretation of these results are confounded by the presence of benzidine, a known human bladder carcinogen. NTP (1986) has tested 3,3'-dimethylbenzidine for carcinogenicity. Preliminary histopathology results indicate that 3,3'-dimethylbenzidine induces a carcinogenic effect in F344 rats when the compound is administered in drinking water (Mennear, 1987). From Griswold et al. (1968) and this data, a potency factor (F) of 34, a potency group of 2, and an EPA Grouping of B2 were determined, which gives 3,3'-dimethylbenzidine a MEDIUM hazard ranking. The documentation supporting this ranking was discussed in Chapter 8. Nevertheless, since IARC (1979) and U.S. EPA (1986a) consider the animal data to be sufficient and the human data to be inadequate, 3,3'-dimethylbenzidine is most appropriately classified as an EPA Group B2 chemical, that is, a possible human carcinogen. When the NTP current study on 3,3'-dimethylbenzidine becomes available, this cancer evidence will be reevaluated and it will be reclassified based on the level of carcinogenic evidence.

10. REFERENCES

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

GLOBAL
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in April, 1986. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

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